

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of Shelley D. Minter

Art Unit 1745

Serial No. 10/617,452

Filed July 11, 2003

Confirmation No. 4859

For ENZYME IMMOBILIZATION FOR USE IN BIOFUEL CELLS AND SENSORS

Examiner Angela J. Martin

DECLARATION OF SHELLEY D. MINTER
UNDER 37 CFR 1.132

TO THE COMMISSIONER OF PATENTS AND TRADEMARKS,
SIR:

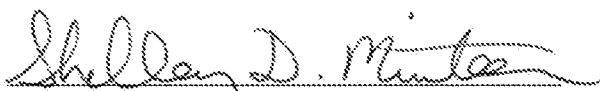
I, Shelley D. Minter, hereby declare and state as follows:

1. I reside at 2150 Gregory, Pacific, Missouri 63069.
2. I received a Doctor of Philosophy in Chemistry from the University of Iowa in 2000.
3. I am currently an Associate Professor of Chemistry at Saint Louis University in St. Louis, Missouri.
4. I am a co-inventor of the subject application, which claims bioanodes and biofuel cells comprising bioanodes.
5. I have reviewed the Office action dated April 3, 2007 in the subject application and studied the Yamamoto et al. reference.
6. I am providing this Declaration to address whether the NADH electron mediator described by Yamamoto is capable of releasing electrons to the electron conductor as required in part (b) of claim 6.

7. Under my direction and control, cyclic voltammograms in 1.0 mM NADH in pH 7.15 phosphate buffer were collected at bare and electrocatalyst modified electrodes as described below.
8. Glassy carbon working electrodes (3mm in diameter, CH Instruments) were polished on a Buehler polishing cloth with 0.05 mm alumina and rinsed in 18 M Ω water. Bare electrodes were dried. Electrocatalyst modified electrodes were polymerized with poly(methylene green). The electrodes were electropolymerized with methylene green by performing cyclic voltammetry using a CH Instrument model 810 potentiostat (Austin, TX) from -0.3 to 1.3 V for 6 scans at a scan rate of 0.05 V/s in a solution containing 0.4 mM methylene green and 0.1 M sodium nitrate in 10 mM sodium borate. The electrocatalyst modified electrode was rinsed and then allowed to dry before testing.
9. All electrodes were allowed to equilibrate in 1.0 mM NADH in pH 7.15 phosphate buffer prior to electrochemical measurements being performed. Cyclic voltammetry was used to measure the electrochemical flux through the membrane. The glassy carbon electrodes (either bare or modified with poly(methylene green)) served as the working electrodes, the reference electrode was a saturated calomel electrode, and the counter electrode was platinum gauze. Data were collected and analyzed on a Dell PC interfaced to a CH Instrument potentiostat model 810. Cyclic voltammetry was performed at 0.10 V/s with cyclic sweeps from -0.5 V to 0.5 V.
10. Attachment A of this declaration shows two cyclic voltammograms, one for a bare electrode and the other for a methylene green modified electrode. The cyclic voltammogram using a bare electrode demonstrates that reduced NADH cannot transfer electrons to the bare electrode as evidenced by the lack of a quantifiable peak upon scanning the pertinent potential range. Therefore, the reduced form of the electron mediator, NADH, did not release electrons to the electron conductor. In contrast, the methylene green modified electrode shows a peak going from negative potential to positive potential and a corresponding decreasing peak current on going from positive potential to negative potential. These peak current changes correspond to

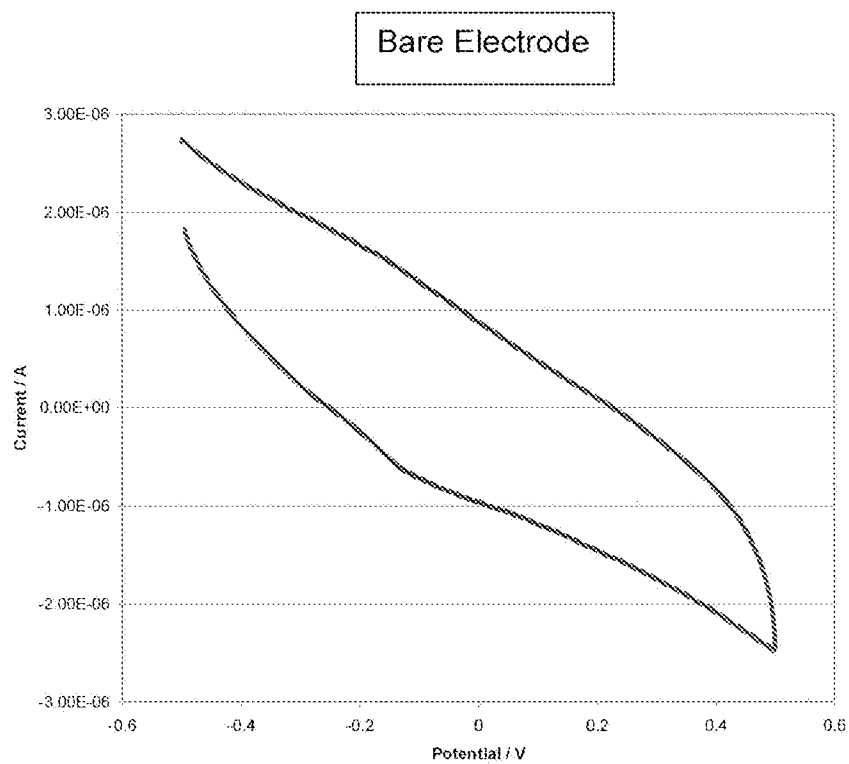
the oxidation of reduced NADH to oxidized NADH and the subsequent reduction of oxidized NADH to reduced NADH. Therefore, the electron mediator NADH transferred electrons to an electrocatalyst (methylene green), and the electrocatalyst in turn released electrons to the electron conductor.

11. I hereby declare and state that all statements made herein are to my own knowledge true; and that all statements made on information and beliefs are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements will jeopardize the validity of the above-identified application or any patent issued thereon.


Shelley D. Minter

9/18/07
Date

Attachment A



Methylene Green modified Electrode

